

## A METHOD FOR TREATING CANCER PATIENTS UNDERGOING CHEMOTHERAPY

### 1. FIELD OF THE INVENTION

The present invention relates to a method for using Product R, a peptide-nucleic acid composition, to treat cancer patients undergoing chemotherapeutic treatments.

### 2. BACKGROUND OF THE INVENTION

Malignant, or cancerous, tumors are defined by their invasion of local tissue and their ability to spread or metastasize to other parts of the body. The incidence of such tumors is high; it is the second leading cause of death in both children and adults. A malignant tumor, by definition, always kills (unless treated) because of its invasive and metastatic characteristics. The tumor grows locally by encroachment into the normal tissues surrounding it. The tumor spreads to distant sites by the off of malignant cells. These cells then move through the blood and lymphatic systems, attach themselves more or less at a remote site, and begin to grow as new colonies.

The factors controlling tumor growth are poorly understood. Tumors in laboratory animals may be transplanted to a second host using only a single tumor cell. This facility suggests that only one normal cell need become transformed (cancerous) for tumor growth to begin. It is thought, however, that many transformed cells die or remain latent or dormant for extended periods before successful tumor growth is established. Tumors have been experimentally induced in animals by chemical, physical, and viral agents, and by radiation and chronic irritation.

Immunological reactions can destroy neoplastic (potentially malignant) cells in vivo, and the accumulation of macrophages within a tumor can lead to its destruction. Cytotoxic T lymphocytes, natural killer (NK) cells, and activated macrophages can kill tumor cells in vitro. These observations suggest that the immune system provides some resistance against the development and spread of cancer, a contention strengthened by increased incidence of spontaneous tumors in individuals with congenital or acquired immune deficiency diseases.

Conventional treatment regimens for tumors include radiation and drugs or a combination of both. All of the conventional anti-cancer drugs are highly toxic and tend to make patients quite ill while undergoing treatment. Vigorous therapy is based on the premise that unless every cancerous cell is destroyed, the residual cells will multiply and cause a relapse.

Most of the conventional chemotherapeutic drugs that are being used in tumor therapy do not specifically kill tumor cells. Reliance is placed on the fact that, in most cancers, the cancerous cells grow faster than normal cells and will therefore utilize more of the toxic chemotherapeutic drug thereby specifically killing the cancer cell. Chemotherapy treatment 5 is given either in a single or in several large doses or, more commonly, it is given in small doses 1 to 4 times a day over variable times from weeks to months. There is a large number of cytotoxic agents used to treat cancer and the mechanisms of the cytotoxic effects of each agent is frequently not known or only partially known. Administration of the conventional chemotherapeutic drugs requires careful attention to the amount and concentration of the drug 10 or combination of drugs so that the cancer cells will be killed but normal cells will survive. For this reason, it is difficult to kill all cancerous cells by conventional chemotherapy. The successful use of chemotherapeutic agents to treat cancer depends upon the differential killing effect of the agent on cancer cells compared to effects on normal tissues.

The effects of chemotherapeutic agents on normal tissues are referred to as side- 15 effects of cancer treatment. The immediate side effects (minutes to a few hours) of chemotherapy may include dizziness, nausea, vomiting, and diarrhea. These side effects are uncomfortable but, in themselves, are not life-threatening. Cell killing or damage within normal tissues that occurs from days to weeks after a commencement of a course of chemotherapy may result in uncomfortable and/or life threatening side effects. Among these 20 effects are hair loss, hearing loss, sterility, damage to the mucosal epithelium of the gastrointestinal tract (namely, GI toxicity), damage to the oral mucosa, esophagus, small and large intestines, kidney damage, skin damage, cardiac damage, killing and suppression of the white blood cells which can lead to infection, reduction of platelets in the blood and killing of hematopoietic blood forming cells. Many of these side effects are related to tissues and organ 25 systems that have a high number of dividing cells (proliferative cells). Some of these side effects are non-life threatening; however, a reduction or prevention of these effects could have a beneficial effect on cancer patients or make it possible to administer a higher dose of the chemotherapeutic agent while minimizing damage or death of cells in normal tissue.

Reticulose® emerged as an antiviral product in the 1930's. While it was originally 30 believed to be a product composed of peptone, peptides and nucleic acids, the precise composition remains unidentified. A method for preparing Reticulose® is provided in U.S. Patent No. 5,849,196, herein incorporated by reference in its entirety. Nevertheless, Reticulose® has demonstrated an ability to inhibit rapidly the course of several viral diseases.

It is nontoxic, miscible with tissue fluids and blood sera and free from anaphylactogenic properties.

As taught by U.S. Patent No. 5,849,196, the components over 15 KDa of the conventional composition of Reticulose® are more effective in treating viral diseases such as 5 HIV, influenza virus, herpes simplex virus, etc. while the components in a range of approximately 1 to 15 KDa function as phagocytosis inhibitors.

Reticulose® suffers from several disadvantages: 1) the method of preparation does not ensure that each preparation produces the finished components in the same ratio, i.e., the final product is not reproducible; 2) the conventional method of preparation produces a wide range 10 of the finished components, which makes the quality control of the preparation extremely difficult, if possible, because too many parameters need to be determined; 3) the presence of the higher molecular weight components, such as 25 KDa component, essentially peptides, increases the risk of hypersensitivity or immune reaction and renders the product less stable. Therefore, it is desirable to have a product devoid of the deficiencies of conventional 15 Reticulose® while maintaining its therapeutic properties.

U.S. Patent Nos. 6,303,153 and 6,528,098, both of which are herein incorporated by reference in their entireties, disclose the preparation of Product R, a composition derived from the same starting materials as used in preparing Reticulose®, but distinct from Reticulose®. For example, material greater than 14 kDa molecular weight is removed when 20 preparing Product R.

Insofar as the applicant knows, Product R has never been used, nor suggested for treating cancer patients undergoing chemotherapeutic treatments. It is now discovered that Product R produces an unexpected result when administered to patients undergoing chemotherapeutic treatments.

25 **3. SUMMARY OF THE INVENTION**

An object of this invention therefore is to provide a method for treating cancer patients taking chemotherapeutic treatments, which reduces the side effects of chemotherapeutic agents on the cancer patients, and/or stimulates the immune system of the cancer patients, by administering to the patients Product R, an antiviral agent composed of 30 peptides and nucleic acids.

The present invention encompasses methods of maintaining or increasing the number of white blood cells in a cancer patient undergoing chemotherapeutic treatment, comprising

administering to said patient an effective treatment amount of Product R. In certain embodiments, the cancer patient does not have basal cell carcinoma or cancer of a lymphocytic cell. In certain embodiments, administering is not subcutaneous, intralesional, topical or by injection. In a preferred embodiment, Product R is administered parenterally in  
5 a sterile injectable formulation. In certain embodiments, the effective treatment amount of Product R is in a range from about 5 microliters to about 40 microliters per kilogram of body weight per day, or from about 10 microliters to about 25 microliters per kilogram of body weight per day, in a sterile formulation. In yet another embodiment, an effective treatment amount of Product R is about 30 microliters per kilogram of body weight per day in a sterile  
10 formulation for about one week, followed by about 15 microliters per kilogram of body weight per day in a sterile formulation.

The present invention also encompasses methods of maintaining or increasing the number of platelets in the blood in a cancer patient undergoing chemotherapeutic treatments, comprising administering to said patient an effective treatment amount of Product R. In  
15 certain embodiments, the cancer patient does not have basal cell carcinoma or cancer of a lymphocytic cell. In certain embodiments, administering is not subcutaneous, intralesional, topical or by injection. In a preferred embodiment, Product R is administered parenterally in a sterile injectable formulation. In certain embodiments, the effective treatment amount of Product R is in a range from about 5 microliters to about 40 microliters per kilogram of body weight per day, or from about 10 microliters to about 25 microliters per kilogram of body  
20 weight per day, in a sterile formulation. In yet another embodiment, an effective treatment amount of Product R is about 30 microliters per kilogram of body weight per day in a sterile formulation for about one week, followed by about 15 microliters per kilogram of body weight per day in a sterile formulation.

25 The present invention also encompasses methods of reducing gastric-intestinal toxicity in a cancer patient resulting from a chemotherapeutic agent, comprising administering to said patient an effective treatment amount of Product R. In certain embodiments, the cancer patient does not have basal cell carcinoma or cancer of a lymphocytic cell. In certain embodiments, administering is not subcutaneous, intralesional, topical or by injection. In a preferred embodiment, Product R is administered parenterally in  
30 an sterile injectable formulation. In certain embodiments, the effective treatment amount of Product R is in a range from about 5 microliters to about 40 microliters per kilogram of body weight per day, or from about 10 microliters to about 25 microliters per kilogram of body

weight per day, in a sterile formulation. In yet another embodiment, an effective treatment amount of Product R is about 30 microliters per kilogram of body weight per day in a sterile formulation for about one week, followed by about 15 microliters per kilogram of body weight per day in a sterile formulation.

5       In specific embodiments, the patient has been administered an anti-cancer chemotherapeutic agent prior to said step of administering Product R.

The present invention further encompasses pharmaceutical compositions comprising an effective treatment amount of Product R, a chemotherapeutic agent, and a pharmaceutically acceptable carrier.

10      The present invention further encompasses kits comprising a first container which contains a unit dosage form of Product R and a second container which contains a chemotherapeutic agent. In certain embodiments, the kit also comprises a needle or syringe.

#### **4. DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS**

15      The present invention provides methods and compositions for treating with Product R cancer patients undergoing chemotherapy. The therapeutic methods of the invention are based on reducing the side effects of chemotherapeutic agents and/or eliciting an immune response in a subject in whom the treatment of cancer is desired, and who has been administered or will be administered a chemotherapeutic agent.

20      As used herein, "about" entails normal experimental variation.

A cancer of a lymphocytic cell includes, but is not limited to, acute lymphocytic leukemia, chronic lymphocytic leukemia, Hodgkin's disease and non-Hodgkin's lymphoma.

##### **4.1 Product R**

25      Product R, a therapeutic composition for treating viral infections and stimulating the immune system, comprises nucleotides and peptides that have molecular weights not more than 14 KDa and substantially not more than 8 KDa. The composition has a light absorption spectrum with typical absorption ratios of 1.998 ( $\pm 10\%$ ) at 260 nm/280 nm and 1.359 ( $\pm 10\%$ ) at 260 nm/230 nm.

30      Product R was used as a synonym of Reticulose<sup>®</sup> in some literature. For the purpose of the present application, Product R and Reticulose<sup>®</sup> represent two distinct products.

Generally, Product R is prepared according to the following manner.

First, the starting materials casein, beef peptone, RNA, bovine serum albumin (BSA), and sodium hydroxide are suspended in proportions of, by weight, 35-50% (casein), 15-40% (beef peptone), 10-25% (RNA), 1-10% (BSA) and 5-25% (sodium hydroxide) in an appropriate volume of distilled water. All starting materials are generally available or otherwise can be readily prepared by a person of ordinary skill in the art. While any RNA is suitable for the intended purpose of the present invention, plant RNA is preferred and yeast RNA is the most preferred. The ratio of total proteins versus the volume of distilled water is generally about 1.5-2.5 to about 100 by weight, preferably about 2.2 to about 100 by weight. This means that every 1.5-2.5 grams of the total proteins are suspended in about 100 milliliters of distilled water.

The suspension as prepared above is then autoclaved at a pressure of approximately 5-15 lbs., preferably 8-10 lbs. under an elevated temperature in a range, for example, from about 150°-300 °F, preferably from about 200° -230 °F, over a period of approximately 2-10 hours, preferably more than 3 hours. As known to a person of ordinary skill in the art, under such conditions RNA may be completely hydrolyzed into nucleotides. After autoclaving, the solution is cooled down to room temperature, and then allowed to stay at a temperature of 3° to 8 °C for at least 12 hours to precipitate insoluble elements. Alternatively, the cooled solution may be centrifuged at a temperature below 8 °C to remove the precipitates.

The resulting solution is then filtered through a 2 micron and a 0.45 micron filters under an inert gas such as nitrogen or argon at a pressure of about 1-6 psi. In a similar manner the solution is filtered again through a pyrogen retention filter, preferably 0.2 micron.

After the above filtration, the solution may be cooled at 3 to 8 °C again for at least about 12 hours and filtered again in the same way as described above.

The resulting filtrate is then assayed for total nitrogen content using methods known to a person of ordinary skill in the art such as Kjeldahl method (Kjeldahl, Z. 1983, Anal. Chem., Vol. 22:366), and its improvements. Based on the assay, the filtrate is then diluted with chilled distilled water to an appropriate volume having a preferred total nitrogen content ranging from 165 to 210 mg/ml.

The pH of the diluted solution is then adjusted with HCl to a physiologically acceptable pH, preferably to about 7.3 to 7.6, after which the diluted solution is filtered again through a 0.2 micron filter under an inert gas as described above.

Product R so produced contains essentially nucleotides, nucleosides and free nucleic acid bases of low molecular weights from a complete hydrolysis of RNA and small peptides

from partial hydrolysis of the proteins. It is possible that the base hydrolysis of the proteins also produces free amino acids.

It is understood that the use of a filtration technique is essentially to remove bacteria or other particles having similar size to or larger size than bacteria. Thus, any filter 5 regardless of its manufacturer or material from which it is made is suitable for the intended purpose. All filters used in the present process are widely available to a person of ordinary skill in the art.

The final filtrate is then filled and sealed into appropriate vials, such as 2 ml or 10 ml glass vials under an inert gas. The filled vials are autoclaved for final sterilization, after 10 which they are ready for use.

An analysis of the composition of Product R reveals that Product R contains two major components, which are exhibited as two bands having molecular weights of 5.2 kDa and 4.3 kDa on a SDS-polyacryamide gel electrophoresis, namely peptide-A and peptide-B, respectively. Peptide-A is a novel single peptide and peptide-B comprises a single peptide 15 covalently bound to an oligonucleotide. Peptide-A and peptide-B are present in the Product R composition in an approximately equal amount and the total amount of these two peptides is about 4.8-5.3 mg/ml, determined by a Lowry protein assay.

The sequence of peptide A is: KVLPVPQKA VPYPQRDMPIQAFLLYQEPVLG (SEQ ID NO. 1). The sequence of peptide B is: GEIPDAGGRIVDYYVGFSDSV (SEQ ID 20 NO. 2). Product R also comprises nucleosides, nucleoside diphosphates and nucleoside monophosphates.

The physical, chemical and biological properties of Product R are further described in U.S. Patent Nos. 6,303,153 and 6,528,098, the contents of which are incorporated by reference in their entirety.

## 25        4.2     Target Cancers

The methods of the invention encompass the use of Product R to treat patients undergoing therapy with chemotherapeutic agents. In specific embodiments, this combination therapy can be used to prevent the recurrence of cancer, inhibit metastasis, or inhibit the growth and/or spread of cancer or metastasis.

30        Product R can be used to treat patients undergoing or that will undergo chemotherapy for the types of cancers that include, but are not limited to human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma,

chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma,  
lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma,  
rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer,  
prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland  
5 carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas,  
cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma,  
hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms'  
tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder  
carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma,  
10 ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendrogioma,  
meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic  
leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic,  
monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic)  
leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's  
15 disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia,  
and heavy chain disease.

In general, Product R is administered to a subject undergoing chemotherapy with one  
or more anti-cancer agents. An anti-cancer agent refers to any molecule or compound that  
assists in the treatment of tumors or cancer. Such anti-cancer agents are well known to those  
20 of skill in the art, and include, but are not limited to, the following categories and specific  
compounds: alkylating agents, antimetabolite agents, anti-tumor antibiotics, vinca alkaloid  
and epidophyllotoxin agents, nitrosoureas, synthetics, and hormonal therapeutic biologics.

Such alkylating agents may include, but are not limited to, nitrogen mustard,  
chlorambucil, cyclophosphamide (cytoxan), ifosfamide, melphalan, thiotepa and busulfan.

25 Antimetabolites can include, but are not limited to, methotrexate, 5-fluorouracil,  
cytosine arabinoside (ara-C), 5-azacytidine, 6-mercaptopurine, 6-thioguanine, and  
fludarabine phosphate. Antitumor antibiotics may include but are not limited to doxorubicin  
(adriamycin), daunorubicin, dactinomycin, bleomycin, mitomycin C, plicamycin, idarubicin,  
and mitoxantrone. Vinca alkaloids and epipodophyllotoxins may include, but are not limited  
30 to vincristine, vinblastine, vindesine, etoposide, and teniposide.

Nitrosoureas include carmustine, lomustine, semustine and streptozocin. Synthetics  
can include, but are not limited to Dacrabazine, hexamethylmelamine, hydroxyurea, mitotane  
procabazine, cisplatin, cisplatinum and carboplatin.

Hormonal therapeutics can include, but are not limited to corticosteroids (cortisone acetate, hydrocortisone, prednisone, prednisolone, methyl prednisolone and dexamethasone), estrogens, (diethylstibesterol, estradiol, esterified estrogens, conjugated estrogen, chlorotiasnene), progestins (medroxyprogesterone acetate, hydroxy progesterone caproate, megestrol acetate), antiestrogens (tamoxifen), aromatase inhibitors (aminoglutethimide), androgens (testosterone propionate, methyltestosterone, fluoxymesterone, testolactone), antiandrogens (flutamide), LHRH analogues (leuprolide acetate), and endocrines for prostate cancer (ketoconazole).

According to the invention, Product R can be administered prior to, subsequently, or concurrently with anti-cancer agent(s), for the treatment of cancer. Depending on the type of cancer, the subject's history and condition, and the anti-cancer agent(s) of choice, the use of the Product R can be coordinated with the dosage and timing of chemotherapy.

Product R may also be used in therapy in conjunction with other medicaments including corticosteroid, gamma globulin, glucose, or vitamins, antiviral agents such as interferon or interleukin, etc.

#### **4.3 Dosage and Administration**

The individual or subject in whom treatment of an cancer is desired is an animal, preferably a mammal, a non-human animal or primate, and most preferably human. The term "animal" as used herein includes but is not limited to companion animals, such as cats and dogs; zoo animals; wild animals, including deers, foxes and racoons; farm animals, livestock and fowl, including horses, cattle, sheep, pigs, turkeys, ducks, and chickens, as well as any rodents.

For the patients having side effects of chemotherapeutic agents or suppressed immune systems caused by chemotherapeutic agents such as, for example, 6-mercaptopurine, adriamycin, bleomycin, cytoxin, chlorambucil, methotrexate, vincristine, 5-fluorouracil, or cisplatin, whether chemotherapeutic agents are employed individually or in any combination, a suitable effective dose of Product R generally will be in the range of from about 5 microliters to about 40 microliters per kilogram of body weight per day, preferably in the range of about 10 microliters to about 25 microliters per kilogram of body weight per day. Most preferably Product R is administered in an amount of about 30 microliters per kilogram of body weight per day for about one week, followed by about 15 microliters per kilogram of body weight per day in a sterile injectable formulation. The desired dose may be

administered as two, three or more sub-doses at appropriate intervals, generally equally spread in time, throughout the day. Preferably, the full daily dose is administered in one administration.

Typical routes of administration for Product R may include, without limitation, oral,  
5 topical, parenteral, sublingual, rectal, vaginal, ocular, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intraperitoneal, intrapleural, intrasternal injection or infusion techniques. In a preferred embodiment, Product R may be administered by any suitable injection route including, but not limited to intravenously, intraperitoneally, subcutaneously, intramuscularly, and intradermally, etc.  
10 Preferably, the compositions are administered parenterally, most preferably intravenously. The presently preferred route of administration is intramuscularly. It will be appreciated that the preferred route may vary with, for example, the condition and age of the recipient.

The efficacy of Product R can be assessed by the maintenance or improvement of white blood cell counts, platelet production, or reduction of gastrointestinal toxicity using  
15 standard methods well known to one of ordinary skill in the art.

#### 4.4 Pharmaceutical Formulations

While it is possible for Product R to be administered as part of a pharmaceutical formulation, it is preferable to present it alone, although it may be administered at about the same time as one or more other pharmaceuticals are independently administered. If Product  
20 R is administered as part of a pharmaceutical formulation, the formulations of the present invention comprise at least one administered ingredient, i.e. Product R, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

25 The formulations may conveniently be presented in unit-dose or multi-dose containers, e.g. sealed ampules and vials.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction of the administered ingredient.

The compositions of the invention can be in the form of a solid, liquid or gas  
30 (aerosol). Pharmaceutical compositions of the invention can be formulated so as to allow a compound of the invention to be bioavailable upon administration of the composition to a subject. Compositions can take the form of one or more dosage units, where for example, a

tablet can be a single dosage unit, and a container of a compound of the invention in aerosol form can hold a plurality of dosage units. A syringe containing a unit dose of Product R is also provided.

#### 4.5 Kits

5 The invention also provides kits for carrying out the methods and/or therapeutic regimens of the invention.

In one embodiment, such kits comprise in one or more containers, Product R.

In another embodiment, such kits comprise in one or more containers therapeutically or prophylactically effective amounts of Product R in pharmaceutically acceptable form.

10 Product R in a container of a kit of the invention may be in the form of a pharmaceutically acceptable solution, *e.g.*, in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, Product R may be lyophilized or desiccated; in this instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (*e.g.*, saline, dextrose  
15 solution, etc.), preferably sterile, to reconstitute Product R to form a solution for injection purposes.

20 In another embodiment, a kit of the invention further comprises a needle or syringe, preferably packaged in sterile form, for injecting Product R, and/or a packaged alcohol pad. Instructions are optionally included for administration of Product R by a clinician or by the patient.

Kits are also provided for carrying out the combination therapies of the present invention. In one embodiment, a kit comprises a first container containing Product R and a second container containing a chemotherapeutic agent for treatment of cancer.

25 The kit may for example comprise metal or plastic foil, such as a blister pack. The kit may be accompanied by one or more reusable or disposable device(s) for administration (*e.g.*, syringes, needles, dispensing pens) and/or instructions for administration.

### 5. EXAMPLES

#### 5.1 Example 1: Method for Preparing Product R

30 Suspend about 35.0 g of casein, about 17.1 g of beef peptone, about 22.0 g of nucleic acid (RNA), about 3.25 g bovine serum albumin in about 2.5 liters of water for injection USP at about 3 to 7 °C in a suitable container and gently stir until all the ingredients have been

properly wet. Carefully add while stirring about 16.5 g of sodium hydroxide (reagent grade ACS) and continue stirring until sodium hydroxide completely dissolved. Autoclave at about 9 lbs pressure and 200 - 230 °F for a period of time until RNA is completely digested, for example, about 4 hours. At the end of the period, the autoclave is stopped and the reaction 5 flask and contents are permitted to slowly cool to ambient temperature. Then cool for at least six hours at about 3-8 °C. The resulting solution is filtered through 2 micron and 0.45 micron filters using inert gas such as nitrogen or argon at low pressure (1-6 psi). In a similar manner the solution is filtered again through 0.2 micron pyrogen retention filters. The resulting filtrate is sampled and assayed for total nitrogen. A calculation is then performed to 10 determine the quantity of cooled water for injection to be added to the filtrate to yield a diluted filtrate with a nitrogen content between about 165-210 mg/100ml, the final volume is approximately 5 liters. The pH is then adjusted with either concentrated HCl (reagent grade ACS) or 1.0 normal NaOH to about 7.3 - 7.6 range. The diluted solution is then filtered again through 0.2 micron filters with inert gas at low pressure. The final filtrate is then filled and 15 sealed into 2 ml glass ampules while in an inert gas atmosphere. The ampules are collected and autoclaved for final sterilization at 240 °F and 20 to 30 pounds pressure for about 30 minutes. Following the sterilization cycle, the ampules with Product R are cooled and washed.

All quantities are subject to plus or minus 2.5% variation for pH, volume, and 20 analytical adjustments.

## 5.2 EXAMPLE 2

Case 1 is that of a 36 year old white male with a history of widely metastatic and progressive malignant melanoma that involved brain, lung, liver, and spleen. He began a regimen of temador (temazolamide) 75 mg/m<sup>2</sup>, and thalidomide 400 mg qhs. He tolerated the 25 treatment with significant side effects — extreme fatigue and weight loss with loss of appetite because of gastric-intestinal (GI) toxicity caused by the chemotherapy. His performance status was KPS 60/100 when Product R 2 cc SC qd was added at his request. Within 24 hours of injection, he reported a substantial improvement in mood, appetite, and willingness to continue with his treatment. He maintained a normal CBC. This went on for 30 approximately 2 weeks. Unfortunately, he then had a bleeding episode from a cerebral metastasis, and was noted to have progressed. He underwent palliative radiation and

subsequently died of complications of pneumonia. He was not able to continue with Product R during his final hospitalization.

### 5.3 EXAMPLE 3

Case 2 is that of a 65 year old white male with a history of widely metastatic bladder cancer involving omentum and mesentery. He suffered from a severe neuropathy related to Taxol chemotherapy and progressed on it after an initial dramatic response. Gemzar was given with further progression and severe GI toxicity, manifested as loss of appetite, and extreme fatigue. Finally as a last attempt at palliation Taxotere with Product R therapy was given. The patient was able to improve his energy level, appetite, and was able to tolerate the Taxotere chemotherapy with no additional neurotoxicity, at essentially average dose (60 mg/m<sup>2</sup>). He remained an actively treated patient and maintained his weight and heme parameters while on Product R, until he died 2 months later of a stroke.

### 5.4 EXAMPLE 4

Case 3 is that of a 34 year old black female with advanced Hodgkin's disease who failed ABVD and stem cell transplant. She was not able to tolerate palliative Navelbine chemotherapy because of extreme fatigue and weight loss. She required doses of between 12-24 mg of dexamethasone to maintain any energy or appetite, and was transfusion dependent. In addition, cervical lymphadenopathy was causing severe pain and discomfort. Product R was added to the Navelbine regimen. Within 2 weeks the patient was maintaining weight, appetite, and although not complete, had an improvement in her ability to maintain a red cell count and platelet count. Because of extensive marrow involvement of the HD, she required intermittent use of growth factors. With the exception of one episode of blood borne sepsis due to an infected Mediport, the patient had been doing well with a significant decrease in overall lymphadenopathy and splenomegaly, based on a CT scan. She died 6 weeks later, when Product R was discontinued, of bacteremia.

### 5.5 EXAMPLE 5

Case 4 is that of a 72 year old male recently diagnosed with acute lymphocytic leukemia. His story is the most dramatic. He arrived in New York on January 10, 2001 after his oncologist in Florida stated that bone marrow done on January 7 showed relapse with 30% blasts. At that point, the dose of Product R which was started 3 weeks before was increased from 2 cc to 4 cc per day. He was profoundly fatigued and had lost 15 pounds. The WBC was 800 with 2000 blasts. Hemoglobin was 11.0, and platelet count was 110,000.

Bactrim given as prophylactic therapy in Florida was discontinued and he was weaned off prednisone. He continued with Product R therapy for approximately 6 weeks of Product R treatment. He last received vincristine at the end of December 2000. He received 3 doses of G-CSF. By the next week his WBC improved and the G-CSF discontinued. Two weeks later  
5 he had a WBC of 10,000 with a normal differential. Hemoglobin was 14.2, hematocrit was 45, and platelet count was 157,000. A rare atypical lymphocyte was seen. A bone marrow aspirate and biopsy were done on January 30, 2001 which revealed an occasional blast cell, as did the biopsy. Normal myeloid lines were seen with mild erythroid hyperplasia. The patient felt great, gained weight, and had improved strength. He entered into a remission  
10 without additional cytotoxic therapy, only taking Product R.

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its  
15 entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be  
20 limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.